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DIAGNOSIS AND PREVENTION OF INFECTION BY PHLEBOTOMUS FEVER GROUP VIRUSES

ANNUAL REPORT

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Expression of the PT N and NS_S proteins has been achieved using baculovirus expression vectors based on AcNPV. High Level expression of both proteins has now been achieved. Expression of the PT M gene products, G1 and G2, in vaccinia vectors has also been achieved.

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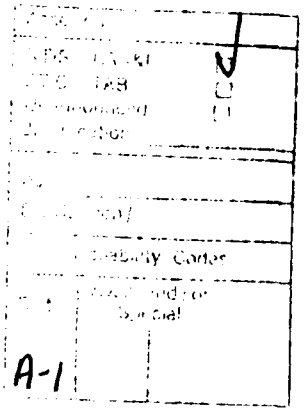


I. SUMMARY

The objectives of the contract are to develop new diagnostic procedures and new vaccine strategies pertinent to selected Phlebotomus fever (PHL) group viruses, viruses that constitute the *Phlebovirus* genus of the negative-sense RNA virus family Bunyaviridae [Bishop *et al.*, 1980]. This genus of viruses includes members that are known animal [including human] pathogens and that are therefore of consequence to military and civilian personnel in particular regions of the world [Bishop & Shope, 1979].

The development of new diagnostic tools for the specific identification of PHL group viruses is being investigated using eukaryotic expression vectors and cDNA clones of particular phlebovirus genes that are available to us. Two expression systems are under study, vaccinia and the insect specific baculovirus expression vectors. So far, it has proven difficult to express the complete M RNA gene products of Punta Toro (PT) phlebovirus using either vector. Recombinant vaccinia viruses have, however, been made that express part of the PT M RNA, producing the PT glycoproteins G1 and G2. The expressed products have been characterized. These studies were undertaken in conjunction with staff of Professor R.W.Compans of the University of Alabama in Birmingham. The expression of PT S RNA gene products supported by this contract and its immediate predecessor [DAMD-17-G-4035, see Overton *et al.*, 1987] using *Autographa californica* nuclear polyhedrosis virus [AcNPV] baculovirus vectors is also reported.

The objective of developing new vaccine strategies for PHL group viruses is being realized through the characterization of an attenuated, candidate vaccine derivative of Rift valley fever [RVF] phlebovirus [the M12 mutant of RVFV isolate ZZ548]. This candidate vaccine was previously developed by staff of the Principal Investigator working at USAMRIID [Caplen *et al.*, 1984]. In this reporting period the complete sequence of the viral M RNA of the M12 RVFV derivative has been obtained.

II. REPORT

A. Introduction

Our analyses have been directed towards developing new strategies for phlebovirus vaccine development. Initial studies, supported by prior U.S. Army Medical Research and Development contracts, were aimed at characterising the

genome and structural components of phleboviruses. With the demonstration of a tripartite RNA genome for phleboviruses, genetic analyses were then undertaken to delineate the coding strategies of the viral RNA species and to determine if recombinant viruses could be obtained and used for vaccine purposes. By analyses of intertypic reassortant PT viruses we initially showed that the viral $7x10^5$ dalton small [S] RNA species codes for the viral $26.9x10^3$ dalton nucleocapsid [N] protein. The results of cloning and sequencing the S RNA of PT phlebovirus [Ihara *et al.*, 1984] confirmed these data and showed that an open reading frame in the *viral-complementary* S RNA sequence coded for N [S *mRNA*]. A *second open reading frame in the viral-sense* strand coding for a $29.1x10^3$ dalton non-structural protein, NS_S [Ihara *et al.*, 1984] was also identified. The two subgenomic PT S induced mRNA species [one viral-complementary, the other viral-sense] were characterized with regard to their 3' and 5' end sequences and rates of synthesis in infected cells [Ihara *et al.*, 1985a; Emery & Bishop, 1987].

The results of cloning and sequencing the $2x10^6$ dalton middle [M] size RNA of PT virus [Ihara *et al.*, 1985b] demonstrated that the PT M RNA codes for the $50\text{-}70x10^3$ dalton viral glycoproteins, G1 and G2. A $30x10^3$ dalton non-structural protein, NS_M, that constitutes the amino terminal end of the PT glycoprotein precursor was also identified. The data showed that the order of the PT M gene products was NS_M-G1-G2. The PT M viral-complementary mRNA species were characterized [Ihara *et al.*, 1985a]. The results obtained for the PT M RNA correlated with the data reported by Collett and associates [1985] for RVFV M RNA and its gene products [RVFV M gene order: NS_M-G2-G1]. Although not proven, it is assumed that the phlebovirus $3x10^6$ dalton large [L] RNA species codes for the $200x10^3$ dalton large protein [a putative transcriptase component] found in viruses.

The genetic studies, including interference assays, reported from the work conducted in the previous contract, indicated that although intertypic reassortant viruses could be obtained, heterotypic virus interactions were not demonstrable (i.e., heterotypic phleboviruses did not interfere with each other and did not reassort their genomes in dual virus infections). Although not all phleboviruses were tested for genetic interactions, the results did not hold out much hope for this approach for vaccine development. Consequently, an alternative approach, that of developing candidate, including subunit, vaccines has been proposed for this contract. These objectives, together with research directed towards the

development of new and specific diagnostic reagents form the principal objectives of this contract.

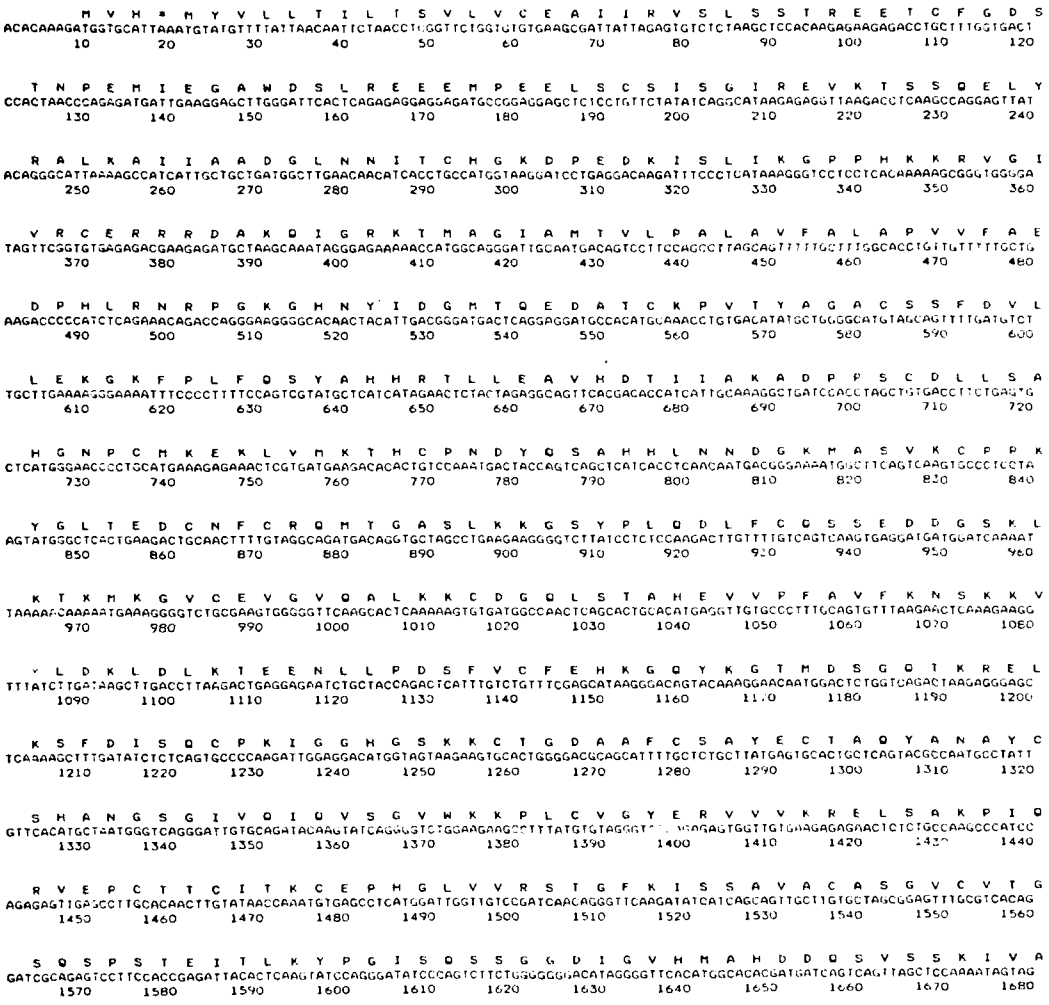
B. Results from this Reporting Period.

The sequence of the M RNA of the M12 candidate vaccine of RVFV

Based on analyses of cDNA clones, the sequence of the M RNA of the ZZ501 strain of RVFV has been reported by Collett and associates (1985). In order to characterize the candidate RVFV vaccine that was produced from the related ZZ548 RVFV [Caplen *et al.*, 1984], we have sequenced the M RNA of the M12 derivative. The sequence was obtained by analyses of λ cDNA clones. The data are shown in Fig. 1 [manuscript in preparation]. Based on the cDNA sequence analyses the RVFV M12 M RNA is deduced to be 3885 nucleotides long (mol. wt. 1.38×10^6, base composition: 27.3% A, 27.2% U, 25.4% G, 20.1% C) and has 3'- and 5'-terminal sequences that are complementary for some 9 residues. The viral RNA codes in its viral-complementary sequence for a single long gene product [the viral glycoprotein precursor] that is comprised of 1197 amino acids (130,000 daltons). The gene product is abundant in cysteine residues but has few potential asparagine-linked glycosylation sites. It is initiated by a methionine codon at nucleotide residues 21-23. These data are similar to those reported for RVFV ZZ501 M RNA [as ammended by recent studies at USAMRIID, J. Dalrymple, personal communication]. However, unlike the ZZ501 sequence, there is a second AUG codon [residues 9-11] in the viral-complementary sequence *upstream* of the M12 M gene product [Fig. 1]. It is followed by valine and histidine codons then a translation terminator [TAA, residues 18-20]. Even discounting this region the 5' non-coding region of the RVFV M viral-complementary RNA is short (20 nucleotides); the 3'-noncoding sequence is much longer (271 nucleotides). No other large open reading frame has been identified in either the viral, or viral-complementary RVFV M RNA sequences.

Limited amino-terminal sequence analyses of the ZZ501 RVFV glycoproteins indicated the gene order and potential cleavage sites in the glycoprotein precursor. The data suggested the existence of a 23×10^3 dalton polypeptide (designated NS_M) in the glycoprotein precursor that precedes the RVFV G2 and G1 proteins (i.e., gene product order: NS_M-G2-G1). Examination of the sequence of the M12 gene product indicates that the ZZ548 M12 and ZZ501 sequences are closely related and that the amino terminal sequences of the viral

Fig. 1. Sequence of the M cDNA of RVFV M12



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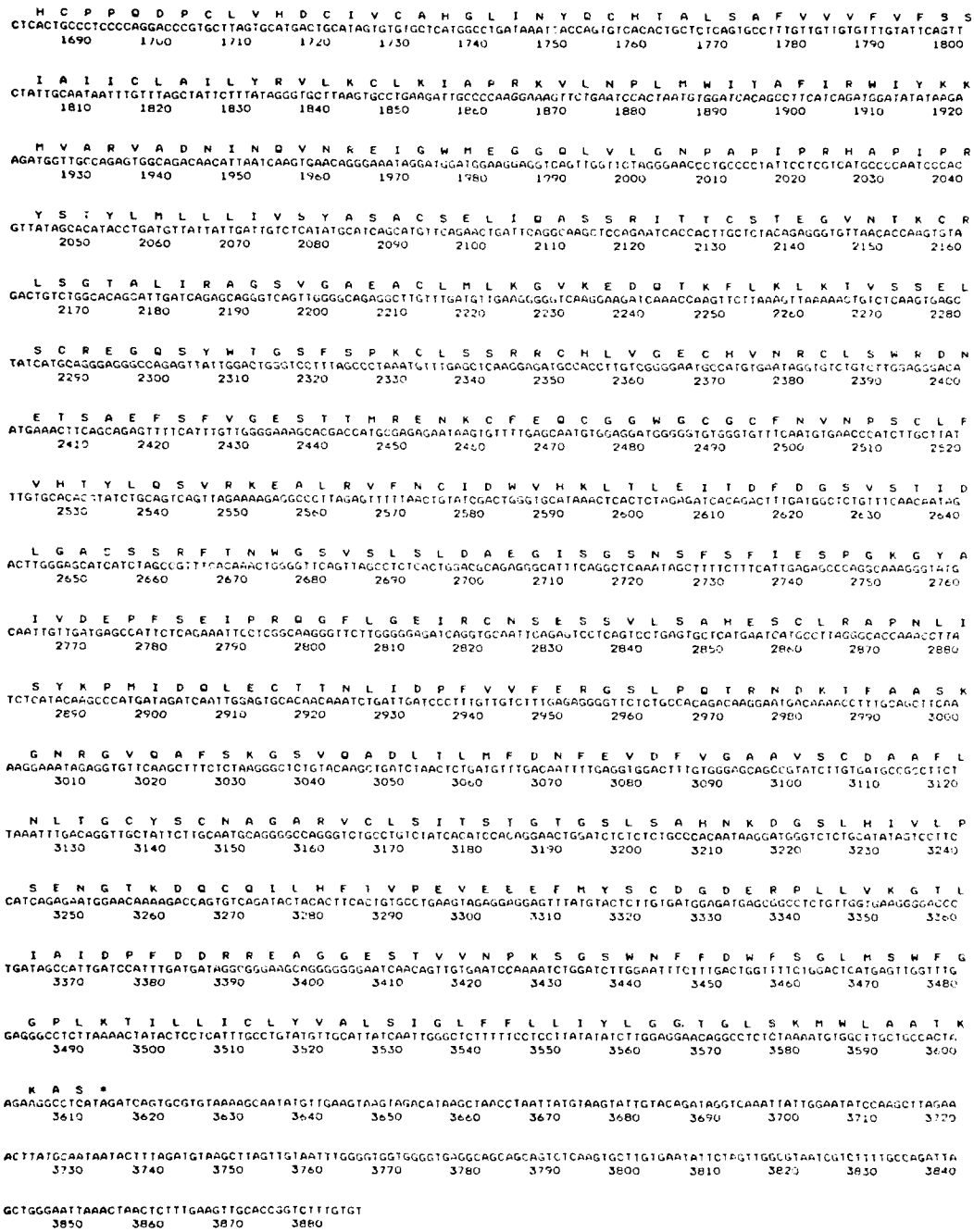

glycoproteins are conserved between them. As shown for PT and the RVFV ZZ501 glycoproteins there are multiple hydrophobic sequences in the M12 M gene product, including a 22-28 amino acid carboxy-proximal, hydrophobic region (G1). This hydrophobic sequence is followed by a 11-amino acid-terminal sequence that contains 3 charged (basic) amino acids. The size and constitution of the carboxy-terminal region is consistent with a transmembranal and anchor function for this glycoprotein in the viral envelope. Other regions of the glycoprotein precursor contain sequences of amino acids with a predominantly hydrophobic character. Their functions are unknown.

Comparison has been made between the reported sequence of ZZ501 M RNA [and its gene product as amended by recent data from USAMRIID] and that of the M12 mutant of ZZ548, recognising that ZZ501 is not the parent of the mutant. Between the two sequences there are XX nucleotide differences and YY amino acid changes. Considering the fact that the M12 virus was derived by consecutive high level mutagenesis of another RVFV strain, these differences are small. Comparison with the sequence of the parent ZZ548 is under analysis at the present time.

Expression of the PT N and NS proteins using baculovirus expression vectors

An essentially complete DNA copy of the ambisense S RNA species of PT phlebovirus [Ihara *et al*, 1984a] has been inserted in either orientation into *Autographa californica* nuclear polyhedrosis baculovirus (AcNPV) in lieu of the 5' coding region of the AcNPV polyhedrin gene [Overton *et al.*, 1987]. The recombinant viruses were generated using what proved to be proficient, but non-optimal expression vectors [e.g., pAcRP6, see below]. The two types of recombinant viruses were used to infect *Spodoptera frugiperda* cells and the expressed PT viral proteins characterised (Overton *et al.*, 1987). Recombinant AcNPV having the S DNA in one orientation expressed PT virus N protein in amounts estimated to represent some 50% of the infected cell extracts, whereas recombinants with the PT S DNA in the other orientation expressed the PT virus NS_S protein in significantly lower quantities. Antisera that were monospecific with respect to each of the two PT proteins virus were raised in mice using the corresponding *S. frugiperda* infected cell extracts and were employed to identify N and NS_S proteins in PT virus-infected Vero cells. These studies demonstrated a

low amount of PT NS_S protein in virions and virion-derived nucleocapsids (Overton *et al.*, 1987).

The results reported previously by this laboratory have now been extended by insertion of each individual gene in a more efficient expression vector derived in our laboratory [pAcYM1, Matsuura *et al.*, 1987]. The level of expression of the PT N protein was similar to that obtained with the pAcRP6 vector, however the level of expression of the NS_S protein was considerably enhanced, approaching that of the N protein. When these analyses are complete they will be prepared for publication.

Expression of PT virus glycoproteins from partial cDNA clones inserted into a vaccinia vector

Partial cDNA clones of the PT M RNA were inserted into the genome of vaccinia virus so that the PT gene products were under the control of an early vaccinia promoter. The PT sequences included in the constructs represented the entire G1 and G2 coding sequences preceeded by some 24-37 upstream amino acids [depending on the constructions]. Recombinant vaccinia viruses were prepared using vaccinia plasmid pSC11 and transfection of CV-1 cells previously infected with strain IHD-J of vaccinia virus. Recombinant viruses were selected by the appropriate procedures [Smith and Moss, 1983]. In CV-1 cells the recombinants expressed glycosylated derivatives of both PT G1 and G2 proteins. In Hela $T4^+$ cells the glycoproteins were identified in the Golgi apparatus. These studies were undertaken in conjunction with Ms Y. Matsuoka and Professor R.W. Compans of the University of Alabama in Birmingham [Matsuoka, Ihara, Bishop & Compans, 1988].

C. Summary of Progress Report

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